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National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Alaska Fisheries Science Center
National Marine Mammal Laboratory
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MEMORANDUM FOR: F/PR1 - Ann Terbush

FROM: F/AKC3 - Doug DeMaster

SUBJECT: Modification of the National Marine Mammal Laboratory's Steller sea lion research permit No. 782-1532-00

The National Marine Mammal Laboratory (NMML) requests modifications to Steller sea lion research permit No. 782-1532-00 in four general areas to augment and improve our field techniques and to incorporate collaborative efforts of colleagues, particularly in Alaska. Most of these requests address evolving research opportunities with collaborating scientists. Other requests adjust for under-estimated takes by disturbance during monthly aerial surveys and scat collection during 2000. Specific modification requests and justifications are as follows:

1. Task 1.b. Authorization for aerial survey during the non-breeding in each year.

The NMML requests the authority to conduct aerial surveys of Steller sea lions during the non-breeding season (August through May) during each year the permit is in effect. As it currently stands, this permit authorizes such surveys up to three times during five years. The initial intent of this task was to conduct Alaska-wide or range-wide surveys during the non-breeding season as many as three times. To date, the NMML has not planned another large-scale survey, rather we are taking advantage of opportunities for cooperative work with colleagues in other agencies. Specifically, biologists with the U.S. Fish and Wildlife Service have recorded observations of Steller sea lions during their aerial surveys for sea otters (*Enhydra lutris*) in the Aleutian Islands and along the Alaska Peninsula. Through this modification, the NMML will be in position to take advantage of such opportunities in the future. Although we request greater frequency for aerial surveys in the non-breeding season, we will not exceed the cumulative number of takes by disturbance authorized under the permit, as each individual survey covers only a relatively small geographical range.

Information about distribution of sea lions outside of the breeding season, particularly in relation to major commercial groundfish fisheries, continues to be of great importance. An additional benefit is that these collaborative surveys, with primary target species other than Steller sea lions, typically focus on different geographical areas or during different times of year than most of the NMML field work. These collaborative efforts do not preclude a future Alaska-wide or range-wide survey under permit No. 782-1532-00.

2. Task 1.c. Increase the number of authorized takes by disturbance and add Southeast Alaska for monthly aerial surveys.

The NMML requests the authority for an increased number of takes of Steller sea lions by disturbance and to add Southeast Alaska for monthly aerial surveys. As it currently stands, permit No. 782-1532-00 authorizes the take of 15,000 sea lions of all ages in the Gulf of Alaska and Aleutian Islands. Prior to monthly surveys in the Kodiak region during the 2000 field season, we estimated that 15,000 takes should be sufficient. Our best estimate for the 2000 activity report of the actual number of sea lions overflown was 16,000. In addition, we plan to conduct similar monthly surveys in Southeast Alaska in collaboration with Dr. Brendan Kelly, a faculty member with the University of Alaska School of Fisheries and Ocean Sciences in Juneau, Alaska. An annual, cumulative take of 35,000 sea lions of all ages will adequately cover the activities in the Kodiak region and in Southeast Alaska.

It is not clear in the existing language of the permit whether "Gulf of Alaska" includes Southeast Alaska. If it does not, we request adding Southeast Alaska to this task, extending authority for monthly surveys Alaska-wide.

3. Task 6. Increase authorized number of takes by disturbance during scat collection.

The NMML requests the authority for an increased number of takes of Steller sea lions by disturbance during scat collection. Permit No. 782-1532-00 currently authorizes the take of 4,000 sea lions of all ages range-wide during scat collection. During the 2000 field season we began a collaborative effort with Ms. Kate Wynne, Research Associate Professor and Marine Mammal Specialist with the University of Alaska, which included regular, seasonal collection of Steller sea lion scats at selected sites in the Kodiak region. The number of takes in the Kodiak region exceeded our estimate, with the result that the cumulative takes for scat collection approached 5,600 animals for all NMML activities Alaska-wide during 2000. In addition to the ongoing collaborative work in the Kodiak region and on-going NMML field expeditions to the Gulf of Alaska and Aleutian Islands, The NMML is collaborating with Dr. Brendan Kelly, of the University of Alaska, on a variety of research projects in Southeast Alaska, particularly in the vicinity of Benjamin Island haulout, near Juneau. Among other activities, Dr. Kelly will collect Steller sea lion scats throughout the year to examine seasonal changes in prey. An annual, cumulative take of 15,000 sea lions of all ages will adequately cover all NMML activities as well as the seasonal scat collection in the Kodiak region and in Southeast Alaska.

The feeding ecology of Steller sea lions, particularly with respect to potential interaction with commercial fisheries, continues to be of great importance. Further, seasonal aspects to diet are becoming increasingly important in assessing potential impacts of commercial fisheries on the status of sea lions. Collection and analysis of scats remains our best technique for identifying the prey of Steller sea lions. The NMML will continue to engage in collaborative efforts to increase both geographic and seasonal coverage in our scat collection program.

4. Task 4. Add additional procedures for animal handling and collection of biological samples.

The requests for modification or addition of procedures to this task will greatly improve our ability to measure sea lion condition, and to further refine measurements of their foraging behavior. Most of these modifications will align the NMML capture and sampling protocol (primarily Prince William Sound westward) to that used by the Alaska Department of Fish and Game (ADF&G) who primarily sample in Prince William Sound and Southeast Alaska, and will allow for complete comparison of data between the two programs. The following modifications/additions pertain to the captures of 120 pups (≥4 months) or juveniles (to three years) currently permitted:

A. We request modification of the permit to allow the optional use of gas anesthesia (isoflurane) to reduce stress on pups greater or equal to 4 months old, and juveniles to three years old (120 animals). Currently the permit allows for this anesthesia technique on pups only, and the optional administration of Valium for the older pups and juveniles. While Valium is appropriate to calm sea lions for a short period of time, the use of gas anesthesia provides sedation for extended periods to allow for additional procedures. We will use the equipment and techniques described in detail by Heath et al. (1996) and Heath et al. (1997). This technique has been used extensively with Steller sea lions of all ages, and was developed for field operations in collaboration with the NMML and ADF&G. Gas will be delivered via mask or by intubation with endotracheal tubes, which is the usual method (Heath et al. 1997). Anesthesia will be applied only by personnel thoroughly trained in its application.

B. We also request modification of the permit to provide authority to hot-brand pups (≥4 months) and juveniles (to 3 years), following the protocol currently approved in the permit for pups. There are two principal reasons for requesting this modification. First, the need for a long-term marking that signifies a sea lion has been captured and sampled. It is becoming clear that it is possible to recapture some sea lions at different ages, particularly through using the underwater capture technique. Such periodic resampling (whether for simple measures of condition, or to deploy instruments) can provide substantial information on growth conditions and the effect of development on foraging behavior. However, without a permanent mark (and flipper tags are not permanent) such an option would not even exist. Secondly, such marked sea lions will contribute to the broader pup branding effort to estimate survival rates, natality rates, and age at first reproduction. Branded animals also will provide information on seasonal movements, dispersal, and site fidelity. The need for a large pool of permanently-marked animals was emphasized by the Recovery Team. To achieve those objectives, intensive resighting efforts will be required. In areas where branding occurs, the NMML will conduct resighting trips in the Spring (approximately April through May), in association with capture trips in the winter (February-March) and summer (August), and during summer branding trips (June-July). Additional resighting efforts will be achieved through field camps at Marmot Island and other locations.

C. The NMML requests authority to add two procedures that will permit calculation of the aerobic dive capacity of juvenile sea lions. One of the focal points of our foraging studies is the development of diving, and hence foraging, ability of juvenile sea lions. Determination of the aerobic dive capacity will provide a better understanding of when Steller's sea lions become physiologically able to access various prey resources. Understanding how this changes during development from pup through juvenile life stages is of critical importance, and will be tied into interpretations of foraging behavior derived from telemetry data. The aerobic dive capacity is partly based upon estimation of oxygen stores available in the blood, muscle and lung. Of those, blood and muscle oxygen stores can be estimated by adding two additional procedures to the those already permitted. These analyses will be conducted by Dr. Jennifer Burns, a collaborator from the University of Alaska at Anchorage:

Total blood volume- Oxygen available stored in blood can be determined by measuring hematocrit and hemoglobin (routinely performed as part of the approved health condition blood sampling protocol), and total blood volume. Total blood volume will be estimated by injection and dilution of Evan's blue dye (ICDH 1973, Foldager and Blomqvist 1991). Evan's blue dye will be injected intravenously at a rate of 3-5cc (0.5 mg/kg body weight), and serial 5 mL blood samples will be collected at 10, 20 and 30 minutes post injection. Evan's blue dye concentration is determined spectrophotometrically. Calculation of total blood oxygen storage capacity is made incorporating measures of blood volume, hematocrit, and hemoglobin concentration (Kooyman et al. 1980; Ponganis et al. 1993).

Muscle biopsies- Muscle oxygen stores are determined by measuring muscle myoglobin content and estimating muscle mass (from deuterated water estimate of lean body mass and allometric equations). Myoglobin content will be determined following methods widely applied to pinnipeds (including Steller's sea lions) without adverse effects or complications (Kanatous et al. 1999; Ponganis et al. 1993; Reed et al. 1994). Additional muscle structure, hormone and enzyme activity analyses will be performed on the samples to examine factors that influence muscle structure and function (Castellini and Somero 1981; Reed et al. 1994; Kanatous et al. 1999). A small muscle biopsy is taken from the major swimming muscle (pectoralis) and the longissimus dorsi (used for terrestrial support and locomotion). On sea lions inducted with gas inhalant anesthesia, sampling sites will be scrubbed with Betadine, and a small incision made with a sterile scalpel blade. A closed muscle biopsy canula needle of 5 mm will be inserted into the incision and pushed through to the muscle layer fascia. When the needle is 1 cm into the muscle, the needle is opened and muscle is forced into the needle using pressure. The needle is then closed and removed, and pressure applied to the site. Two 25-35 mg samples are taken from each site at different angles. Two samples are necessary because myoglobin assays require flash frozen tissues, while the fiber type assays require fixed tissue. Biopsy needles will be sterilized with a cold liquid sterilizer between animals. It has been found that sutures are not required for wound healing.

D. One of the principal elements of this task is to monitor the health, condition, and vital parameters of sea lions, and to develop indices of condition using non-lethal techniques as identified in the Recovery Plan (NMFS 1992, research tasks 4 and 432). Currently Permit No. 782-1532-00 authorizes the collection of mass, girth and length as condition indices for older pups and juveniles, and collection of blood and fecal samples from those animals for additional health assessment. This combination of morphometric measurements (e.g., mass, length, girth) provide two principal indices of condition, a body mass index (mass corrected for body length or sampling date) and girth index (girth corrected for length). If combined with an age determination (requested modification 4.F.), mass or length at age provide invaluable measures of conditions experienced by that sea lion since birth. However, determining the body composition of a sea lion, and therefore the proportion of the body that is fat, provides a more complete picture of condition. The NMML requests authority to add deuterated water (D2O) injection and bioelectrical impedance analysis for up to 120 pup/juveniles captured under Task 4. Addition of these measures will provide for direct comparisons to sea lions capture by ADF&G from other parts of the range. Sea lions under gas anesthesia (see requested modification Task 4.A.) will be given an intramuscular injection of known volumes (1 g D2O/kg body mass) of deuterated water (99% enriched, Metabolic Solutions, Inc.). Equilibration of the isotope of two hours will occur while other procedures are being performed. A pre-final sample of serum will be drawn 20 min prior to the final sample, at 2 hours. Mass spectrophotometric analysis of D2O in water distilled from blood samples will be conducted by a commercial laboratory (Metabolic Solutions, Inc.). Calculation of percent body fat will be made using equations from Bowen and Iverson (1998), and assuming a hydration of 0.73 (Wang et al. 1999).

The NMML also seeks authority to add bioelectric impedance analysis (BIA) as an optional procedure for the 120 pup/juveniles. A rapid measure of body composition is possible using a bioelectric impedance analyzer (BIA). The conductivity of a whole body is related to the distribution of water and electrolytes, and by modeling the whole body as a conductor measurements of reactance and resistance can be converted into estimates of body fat (Lukaski 1987). This requires developing a mathematical relationship between values determined from BIA, and another measure of body composition, such as deuterated water dilution. This technique has been widely applied in marine mammals, with varying degrees of precision (Gales et al. 1994; Arnould 1995; Bowen et al. 1998; Bowen et al. 1999). Currently this technique looks very promising for assessing individual condition in Steller's sea lions. A recent analysis by Castellini (2001) found an excellent relationship between deuterated water measures of total body water and BIA impedance measurements. Adding this determination to our methodology will expand the data used in creating the model. The procedure is simple and quick. On a sedated sea lion, four 1 ½ inch 20 G needles are inserted subcutaneously (two anterior just behind the skull, two posterior near the tail) as electrodes. Leads from these electrodes attach to a portable BIA unit (RJL Enterprises Quantum II, or Model 101A) and instantaneous readings of resistance and reactance are obtained. The electrodes are then removed. For best precision, the measures are repeated 2-5 times. Ultimately this technique may replace deuterated water injection as a method to determine body

composition, particularly in situations where waiting for the lengthy equilibration period is unfeasible.

E. To accommodate procedures requiring additional blood samples, the NMML requests that on older pups and juveniles the total allowable blood volume withdrawn during a handling be increased from the currently permitted 40 mL to 120 mL. This increased volume would only be drawn from sea lions subjected to the combination of routine blood sampling, Evans blue dye injection, and deuterated water injection. All efforts will be made to minimize the amount drawn by combining blood analyses as appropriate. The mass of these animals is 80-120 kg. Pediatric human phlebotomy guidelines recommend up to approximately 1.2 mL/kg as a maximum volume to be withdrawn at any one time (Garza and Becan-McBride 1984). However, otariids have a much greater blood volume per body mass than do humans (Kooyman 1985). Thus, the requested increase still results in an extremely conservative withdrawal rate. This increased volume will allow for the greater range of analyses without physical jeopardy to the animals.

F. One of the most useful measures of body condition is the mass or size of a sea lion relative to its age. The ages of pups and juveniles captured under the current permit are estimated based on a combination of teeth eruption pattern, general animal size, and season of capture. This technique becomes much less precise after a sea lion is older than one year. We therefore seek approval to add tooth extraction for the purposes of age determination of juveniles, in cases where age is indeterminable by the above criteria. Following the protocol approved for ADF&G sea lion captures, such extractions of one 2nd pre-molar tooth from the right side would be accomplished by use of a scalpel to loosen attachments, and then extracted with a dental elevator on sea lions under general gas anesthesia. Sea lion age can then be determined by counting incremental growth layers on a longitudinal section using standard procedures at the NMML laboratory.

G. Under the current permit, one or two vibrissae may be clipped close to the skin from pups (≥4 months old) and juveniles (up to 3 years old) for stable isotope analysis to help identify the general trophic level at which an animal is feeding over prolonged periods. The NMML requests the permit be modified to allow pulling, rather than clipping of vibrissae to align our collection protocol to that approved for use by ADF&G. Clipping a vibrissae results in an unknown length remaining attached to the sea lion. Stable isotope ratios show regular, oscillating patterns in Steller's sea lion vibrissae of 1-3 cm, and changes in ratios can occur in less than 1 cm (Hirons et al. 1998). Thus, obtaining the root of the vibrissae, representing the most recent growth, for analysis is crucial. Following approved ADF&G protocol, a vibrissae would be pulled by gripping with forceps and pulling forcefully and rapidly in one smooth motion.

H. The NMML currently has authority under this permit to attach satellite-linked time depth recorders (SLTDR) and VHF transmitters to pups (≥4 months old) and juveniles (to 3 years) for determination of habitat requirements and investigations of feeding ecology. We seek additional authority to deploy newly available Underwater Timed Picture Recorders (UTPR) on some of the 120 sea lions authorized for instrument deployment. These instruments, developed by Wild Insight Ltd., are 55 x 85 x 105 mm, weigh 700 g on land and 200 g in water, were designed to facilitate studies of how animals interact with the environment, their prey, and other animals at depth. The UTPRs can record up to 3 hours of still, time lapse, or movie images, and are integrated with an incorporated Wildlife Computers Mark 7 time-depth recorder (TDR) to record dive behavior and start/stop image recording according to program directions. The NMML would deploy an UTPR in conjunction with a VHF transmitter and optionally a simple PTT (Sirtrack or Wildlife Computer SPOT tag). In combination, this will provide the most detail possible for location, dive behavior, and prey interaction currently available. VHF and PTT transmitters would be attached following protocols currently in the permit. The UTPR would be attached with a remotely release (by radio signal) platform following attachment protocols for SLTDRs already in the permit. UTPRs would thus be retrieved without a recapture of the sea lion required.

If you have questions or need more information, please contact or Tom Loughlin (206-526-4040), Brian Fadely (206-526-6173), or John Sease (206-526-4024).

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